* NOTICES *

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] This invention relates to the method of predicting the stability when forming oligo ribonucleotide, complementation [it] RNA oligodeoxyribonucleotide or DNA, and complementary base pair structure.
[0002]
[Description of the Prior Art] It is important to predict the stability when forming oligo ribonucleotide, complementation [it] RNA oligodeoxyribonucleotide or DNA, and complementary base pair structure, i.e., the strength of association, from viewpoints, such as development of antisense-nucleic-acid physic, and development of the diagnosis / detection method which used the nucleic acid as the target.
[0003] Prediction of the stability of the complementary base pair structure of oligo ribonucleotides Oligo ribonucleotides complementary base pair structure The maximum contiguity base pair parameter when forming It can carry out by using. [S.M.Freier et al. and Proc.Natl. 83 Acad.Sci.USA, 9373 - 9377 pages (1986); D.H.Turner, N.Sugimoto, S.M.Freier, Ann.Rev.Biophys.Biophys.Chem.17 volume, and 167 -192-page (1988)], [Naoki Sugimoto permitted the usefulness, living thing physics 33 volumes, 61 -67-page (1993)].
[0004] Prediction of the stability of the complementary base pair structure of oligodeoxyribonucleotides can be similarly performed using the maximum contiguity base pair parameter in case oligodeoxyribonucleotides form complementary base pair structure [K.J.Breslauer et al., 83 Proc.Natl.Acad.Sci.USA, and 3746 - 3750 pages (1986)].
[0005]
[Problem(s) to be Solved by the Invention] However, although there is the method [30 Biochemistr(ies) and 1097 - 1118 pages (1991)] of T.D.Yager and P.H.von Hippel about prediction of the stability of the complementary base pair structure of oligo ribonucleotide and oligodeoxyribonucleotide, the forecast which the parameter proposed there is not based on the experiment fact, and all asked for it by this method has the large gap from an experimental value. This invention aims at offering the method of predicting structural stability in case oligo ribonucleotide and oligodeoxyribonucleotide [complementation / it] form complementary base pair structure with high degree of accuracy.
[0006]
[Means for Solving the Problem] In this invention, it asks for change of enthalpy accompanying formation of complementary base pair structure of oligo ribonucleotide and oligodeoxyribonucleotide which has a complementary base sequence in it, entropy, or free energy using the maximum contiguity base pair model, and the structural stability of complementary base pair structure of the length of arbitration of RNA/DNA is predicted based on the change. Stability can be predicted by change (it is stability, so that an absolute value is large at a value negative in enthalpy change) of enthalpy accompanying formation of for example, complementary base pair structure, entropy change (it is stability, so that an absolute value is large at a value positive in entropy change), and free energy change (it is stability, so that an absolute value is large at a value negative in a free energy change). or [that such change takes place especially] -- a free energy change is decided by whether it is taking a value negative in whether it being ****** (such change taking place, when taking a negative value).
[0007] N1N2 ... Change of enthalpy accompanying formation of complementary base pair structure of oligo ribonucleotide expressed with Nn and oligodeoxyribonucleotide which has a complementary base sequence in it, entropy, or free energy is called for according to a degree type (1).
[0008]

$$\sum_{i=1}^{n-1} \{X\ (N_i N_{i+1})\} + Y \qquad (1)$$

[0009] However, nickel (n: [i= 1, 2, --,] n is a positive integer) Either of four sorts of bases A, G, C, and U which constitute oligo ribonucleotide is expressed. X (NiNi+1) (i= 1, 2, --, n-1) They are enthalpy accompanying complementary base pair structure formation with oligodeoxyribonucleotide of a dimer complementary to a base sequence of an oligo ribonucleotide NiNi+1 and this nucleotide of a dimer, entropy, or the value of a parameter to change of free energy. The sum shown by sigma means taking the sum of an individual (n-1) of X (N1N2), X (N2N3), --, X (Nn-1Nn). Moreover, Y is the correction term (initiation) of enthalpy relevant to the first base pair generation, entropy, or free energy.
[0010] In this invention, a value shown in a table 1 is used as a value of enthalpy change (deltaH degree shows) to 16 kinds of a JIRIBO nucleotide / dideoxy ribonucleotide base pairs, and an initiation factor effective in the maximum contiguity base pair model, entropy change (deltaS degree shows), and a free energy change (deltaG degree37 show). In a table 1, r expresses ribonucleotide, d expresses deoxyribonucleotide, in how to locate a base in a line, the start expresses a five prime end side and the back expresses a

three-dash terminal side. Moreover, for an unit of a value shown in a table 1, deltaH degree . kcal mol-1 and deltaS degree cal mol-1k-1 and deltaG degree37 It is kcal mol-1.
[0011]
[A table 1]
** Train deltaH degree deltaS degree deltaG degree37 ---------- rAA/dTT - 7.8 - 21.9 - 1.0 rAC/dGT - 5.9 - 12.3 - 2.1 rAG/dCT - 9.1 - 23.5 - 1.8 RAU/dAT -8.3 - 23.9 - 0.9 RCA/dTG -9.0 - 26.1 - 0.9 rCC/dGG -9.3 -23.2 - 2.1 RCG/dCG -16.3 - 47.1 - 1.7 RCU/dAG - 7.0-19.7 - 0.9 RGA/dTC - 5.5 -13.5 -1.3 RGC/dGC -8.0-17.1 -2.7 rGG/dCC -12.8-31.9-2.9 rGU/dAC -7.8-21.6 - 1.1 RUA/dTA -7.8 - 23.2-0.6 rUC/dGA - 8.6 -22.9 - 1.5 rUG/dCA - 10.4 -28.4-1.6 RUU/dAA -11.5 -36.4 -0.2 Open ** 1.9 -3.9 3.1
[0012] Moreover, whether it uses a value of a range shown in the following table 2 instead of a value shown in a table 1 or uses a value of **10% of within the limits of a value shown in a table 1, although precision falls a little, it can predict the stability of complementary base pair structure of oligo ribonucleotide / oligodeoxyribonucleotide in precision which should be satisfied enough.
[0013]
[A table 2]
** Train deltaH degree deltaS degree deltaG degree37 ---------- rAA/dTT - 8.1--7.5 - 23.2--20.6 - 1.1--0.9 rAC/dGT - 6.2--5.6 - 13.6--11.0 - 2.2--2.0 rAG/dCT - 9.4--8.8 - 24.8--22.2 - 1.9--1.7 RAU/dAT - 8.6--8.0 -25.2--22.6-1.0--0.8 rCA/dTG - 9.3--8.7-27.4--24.8-1.0 - -0.8 RCC/dGG - 9.6--9.0-24.5--21.9 - 2.2 - -2.0 RCG/dCG -16.6--16.0 -48.4--45.8 - 1.8--1.6 RCU/dAG-7.3--6.7 - 21.0--18.4 - 1.0--0.8 rGA/dTC -5.8--5.2 - 14.8--12.2 - 1.4--1.2 RGC/dGC-8.3--7.7 - 18.4--15.8 - 2.8--2.6 RGG/dCC -13.1--12.5 -33.2--30.6 - 3.0--2.8 rGU/dAC - 8.1--7.5-22.9--20.3 - 1.2--1.0 RUA/dTA - 8.1--7.5-24.5--21.9 - 0.7--0.5 rUC/dGA -8.9--8.3 -24.2--21.6 - 1.6--1.4 rUG/dCA -10.7--10.1 -29.7--27.1 - 1.7--1.5 RUU/dAA -11.8--11.2 -37.7--35.1 -0.3--0.1 Open ** 1.6-2.2 -5.2--2.63.0-3.2
[0014]
[Example] Hereafter, although an example explains this invention still more concretely, this invention is not limited to these examples. The oligo ribonucleotide and oligodeoxyribonucleotide which consist of five to 12 base used as a sample it compounded by the phosphoroamidite method using a solid phase technique (F.Eckstein -- editing --) Oligonucleotides and Analogues: A Practical Approach, IRL Press, 1991; The volume on S.Agrawal, and 20 Methods in Molecular Biology Protocols for Oligonucleotides and Analogs, Humana Press, 1993. Purification and demineralization of the sample obtained by composition were done using the opposition type sep pack cartridge (a C-18 Sep-Pak cartridge, product made from Waters), and it was used for the following experiments.
[0015] The presentation became the buffer solution from 1MNaCl/10mMNa2HPO4/1mMNa2EDTA, and that the acidity or alkalinity of whose is pH7.0 was used for it. The concentration of oligo ribonucleotide and oligodeoxyribonucleotide Molar extinction coefficient [E.G.Richards of the mononucleotide reported and dinucleotide, Handbook of Biochemistry and Molecular Biology : [Nucleic] Based on Acids (C.D.Fasman ed.) 3rd ed., Vol.I, p197, and CRC Cleveland OH.] According to the method [N.Sugimoto et al., 25 Biochemistr(ies), and 5755 - 5759 pages (1986)] using the likeness method of approach, it determined at 90 degrees C recently. Oligo ribonucleotide and oligodeoxyribonucleotide [complementation / it] were mixed so that each concentration might become the same in the buffer solution.
[0016] The solution which contains oligo ribonucleotide and oligodeoxyribonucleotide [complementation / it] the said concentration every was put in into the above-mentioned buffer solution at the cel for ultraviolet radiation measurement, and the absorbance in an ultraviolet radiation field was measured with the spectrophotometer (the Hitachi make U-3200 and U-3210). In order to investigate the temperature dependence of meeting / dissociation condition of the complementary nucleic-acid chains contained especially there, the absorbance in the various temperature in 260nm of the buffer solution containing a sample was pursued changing temperature at per minute 0.5 or a rate of 1.0 degrees C. Under the present circumstances, Hitachi SPR-7 or a SPR-10 temperature programmer was used. Moreover, adhesion of waterdrop was prevented by pouring desiccation nitrogen if needed. The obtained data was optimized by the suitable method using PC-9801 computer by NEC Corp., and the melting out temperature (T<SUB>m) was determined as after an appropriate time according to the already reported method [M.Petersheim and D.H.Turner, 22 Biochemistr(ies), 256 -263-page (1983); N.Sugimoto et al., and Chem.Lett.9-12 page (1991)].
[0017] and the degree type by Turner and others -- the enthalpy change (it expresses with deltaH degree) and entropy change (it expresses with deltaS degree) accompanying formation of complementary base pair structure were computed using (2 [D.H.Turner et al. and Landolt-Bphirnstein Nucleic Acids 1 (volume on W.R.Saenger) c s chapter 3.6 Berlin Germany (1990)]). Inside Ct of a formula The total concentration of oligo ribonucleotide and oligodeoxyribonucleotide and R are gas constants.
Tm-1={Rln(Ct/4)+deltaS degree} / deltaH degree (2)
That is, the value (deltaH degree and deltaS degree) was computed by plotting the value of Tm-1 to the value of ln (Ct/4). Moreover, the value of the free energy change (it expresses with deltaG degree37) in 37 degrees C was calculated by assigning a value (deltaH degree and deltaS degree) to a degree type (3).
deltaG degree37=deltaH degree-(37.0+273.15) deltaS degree (3)
[0018] It investigated about the case so that the base pair of the JIRIBO nucleotide which constitutes it, and dideoxy ribonucleotide may not change to the beginning even if it replaces the array sequence of the base pair of oligo ribonucleotide and oligodeoxyribonucleotide [complementation / it] in order to check that it is effective in the duplex chain formation which the idea of the maximum contiguity base pair becomes from oligo ribonucleotide and oligodeoxyribonucleotide [complementation / it]. For example, r (CUUACGCU)/d (AGCGTAAG)
since -- r (CUACGCUU)/d [chain / duplex / of an octamer / becoming / [the start is 5' edge and r (CUUACGCU) and d (AGCGTAAG) is / the last / 3' edges here]] (AAGCGTAG)
since -- both the JIRIBO nucleotides or dideoxy ribonucleotides that constitute them are expressed in the following seven sorts as the becoming duplex chain of an octamer.
rCU/dAG rUU/dAA rUA/dTA rAC/dGT rCG/dCG rGC/dGC rCU/dAG [0019] Here, r expresses ribonucleotide, d expresses deoxyribonucleotide, the start expresses a five prime end side and, as for how to locate a base in a line, the back expresses the three-

dash terminal side. Drawing 1 plots the result obtained in the experiment according to (2) types about the duplex chain of an octamer which consists of r (CUUACGCU)/d (AGCGTAAG), and the duplex chain of an octamer which consists of r (CUACGCUU)/d (AAGCGTAG). That is, about the case where it carries out by various oligo ribonucleotide concentration or various oligodeoxyribonucleotide concentration, when those total concentration is set to Ct, the value of the common logarithm of Ct/4 is plotted to the value which doubled the inverse number of a melting out temperature 1000. Among drawing, (<>) is the plot to r (CUACGCUU)/d (AAGCGTAG), and (+) is the plot to r (CUUACGCU)/d (AGCGTAAG).

[0020] By drawing 1 and the above (2), and (3) types, when the deltaH degree, deltaS degree, deltaG degree37, and Tm were calculated about each, it did not pass over both difference respectively at 3.8%, 3.3%, 6.1%, and 2.7 degrees C, but it was very small. In addition, both deltaH degree, deltaS degree, deltaG degree37, and Tm were measured and compared about five pairs of duplex chains with which structure is similar. Those results are collectively shown in a table 3. For the unit of each parameter, deltaH degree . kcal mol-1 and deltaS degree cal mol-1K-1 and deltaG degree37 kcal mol-1 and Tm are **s.

[0021]

[A table 3]

Pair number Hybrid duplex chain deltaH degree deltaS degreedeltaG degree37 Tm ---------- [1] r (CUACGCUU)/d (AAGCGTAG) -54.3 - 153 - 6.8 38.9 r (CUUACGCU)/d (AGCGTAAG) -52.3 - 148 - 6.4 36.2 [2] r (ACCGCA)/d (TGCGGT) - 45.4-126 - 6.4 36.1 R (GCACCG)/D (CGGTGC) -49.7 - 141 - 6.2 34.8 [3] r (CACGGC)/d (GCCGTG) - 48.9 -135- 6.939.5 R (GGCACG) / D (CGTGCC)-51.8 - 144 - 7.3 41.5 [4] r (AAGCGUAG)/d (CTACGCTT) -67.2 - 192 - 7.8 43.1 r (AGCGUAAG)/d (CTTACGCT) -60.1 - 170 -7.3 41.1 [5] R (AGUCCUGA)/D (TCAGGACT) -54.7 - 155-6.7 37.9 r (CUGAGUCC)/d (GGACTCAG) -60.8 -171-7.843.4 [6] r (GAGCCGUG)/d (CACGGCTC) -67.3 -187 -9.5 51.4r (GCCGUGAG) / d (CTCACGGC)-71.4 -199 -9.7 51.4 [0022] For **4% delta S degrees, **4% delta G degree of a measurement error of Tm(s) is [deltaH degree / 37] **2% **8%. The melting out temperature Tm calculated the total concentration of oligo ribonucleotide and oligodeoxyribonucleotide as 100microM. Like [it is ****** from a table 3 and], the difference between deltaH degree in each set of [1] - [6], deltaS degree, deltaG degree37, and Tm is seen on the average, and does not exceed an experimental error greatly. These results show that the maximum contiguity base pair model is effective also in the case of oligo ribonucleotide / oligodeoxyribonucleotide.

[0023] Thus, after checking the effectiveness of the maximum contiguity base pair model, the value of deltaH degree, deltaS degree, deltaG degree37, and Tm over each base pair formation was measured by the same method as the above by the above-mentioned method about the complementary base pair of 64 kinds of oligo ribonucleotide / oligodeoxyribonucleotides. A result is shown in a table 4. moreover, the inside of a table -- the measured value of six sorts of last RNA arrays from "CACAG" to "GUUGGUUGGUUG" -- Hall et al.Biochemistry 30 and 10606-10613 (1991) -- and -- Ratmeyer et al.Biochemistry 30 and 5298-5304 (1994) It depends. About the numeric value in the parenthesis in a table 4, it mentions later. For the unit of each numeric value, deltaH degree . kcal mol-1 and deltaS degree cal mol-1K-1 and deltaG degree37 kcal mol-1 and Tm are **s. In addition, the left column of a table 4 shows only the oligo ribonucleotide which forms a complementary base pair. Following [for example,], the numeric value of the 1st line of the 4th table expresses change of each parameter at the time of r(AGCCG)/d (CGGCT) formation.

[0024]

[A table 4]

Oligo ribonucleotide array deltaH degree deltaS degree deltaG degree37 Tm ---------- AGCCG - 41.6 (-40.8) - 116 (-115) -5.7 (-5.2) 30.8 (27.2) CGGCU - 45.8 (-42.2) -132 (-120) -4.9 (-5.1) 26.1 (26.7) GGUGG - 46.0 (-41.9) -132 (-118) -5.2 (-5.4) 28.0 (28.8) ACCGCA-45.4 (-46.6) -126 (-130) -6.4 (-6.4) 36.1 (36.0) CAAUCG - 46.7 (-48.1) -140 (-146) -3.3 (-2.9) 16.9 (15.1) CACGGC - 48.9 (-50.1) -135 (-138) -6.9 (-7.2) 39.5 (41.0) CGAUUG - 49.3 (-50.1) -151 (-153) -2.6 (-2.6) 14.1 (14.4) CGGUGC - 48.9 (-53.4) -135 (-150) -7.0 (-6.9) 40.1 (39.0) CGUGCC - 49.3 (-49.9) -139 (-141) -6.3 (-6.1) 35.3 (34.2) GCACCG - 49.7 (-46.6) - 141 (-130) -6.2 (-6.4) 34.8 (36.0) GGCACG - 51.8 (-50.1) -144 (-138) -7.3 (-7.2) 41.5 (41.0) AAUACCG - 54.1 (-53.5) -160 (-156) - 4.7 (-5.3) 26.6 (29.9) ACGUAUG - 58.3 (-54.6) -172 (-160) -5.0 (-4.9) 29.6 (27.8) AGCUUCA - 55.6 (-51.3) -164 (-150) -4.7 (-4.9) 26.2 (27.5) CACGGCU - 53.9 (-57.1) -147 (-158) -8.5 (-8.1) 48.6 (45.6) CAUACGU - 53.2 (-53.2) -158 (-158) -4.3 (-4.2) 24.5 (23.8) GGACUUA - 43.8 (-48.6) -125 (-141) -4.9 (-4.9) 25.9 (26.9) UAAGUCC - 46.2 (-48.5) -132 (-140) -5.4 (-5.0) 29.4 (27.6) UGAAGCU - 43.0 (-45.9) -118 (-128) -6.4 (-6.2) 36.5 (34.8) AAAAAAAA - 54.0 (-52.7) -162 (-157) -3.8 (-3.9) 22.1 (22.5) AAGCGUAG - 67.2 (-64.0) -192 (-182) -7.8 (-7.6) 43.1 (42.3) AAUCCAGU - 55.9 (-58.0) -161 (-167) -6.1 (-6.2) 34.5 (35.3) AAUGUCGC - 64.9 (-65.3) -186 (-187) -7.2 (-7.4) 39.9 (41.0) ACCUAGUC - 56.0 (-53.6) -159 (-150) -6.9 (-7.0) 39.2 (39.7) ACGACCUC - 55.0 (-56.6) -150 (-155) -8.6 (-8.6) 48.9 (48.5) ACUGGAUU - 57.8 (-59.5) -163 (-170) -7.2 (-6.8) 40.9 (38.3) AGCGUAAG - 60.1 (-64.0) -170 (-182) -7.3 (-7.6) 41.1 (42.3) AGUCCUGA - 54.7 (-55.8) -155 (-157) -6.7 (-7.2) 37.9 (40.8) CAACAGCA - 52.7 (-55.9) -145 (-157) -7.8 (-7.2) 44.8 (40.8) CACGGCUC - 71.6 (-65.7) -200 (-181) -9.6 (-9.6) 50.5 (52.0) CGCUGUAA - 60.4 (-63.2) 38.9 (34.2) -172 (-183) -7.2 (-6.5) 40.2 (36.7) CUACGCUU -54.3 (-61.6) -153 (-179) -6.8 (-6.0)) CUAGUGGA -63.4(-58.5) -178(-166) -8.3(-7.1) 45.1(40.1) CUCACGGC -70.3(-65.7) -196(-181) -9.6(-9.6) 51.0(52.0) CUGAGUCC -60.8(-55.8) -171(-157) -7.8(-7.2) 43.4(40.8) CUUACGCU -52.3(-61.6) -148(-179 -6.4 (-6.0) 36.2 (34.2) GACUAGGU - 57.0 (-54.0) -158 (-150) -8.1 (-7.6) 45.9 (43.3) GAGCCGUG - 67.3 (-64.5) -187 (-178) -9.5 (-9.2) 51.4 (50.4) GAGGUCGU - 71.9 (-66.0) -202 (-186) -9.2 (-8.3) 48.9 (45.6) GCCAGUUA - 62.9 (-60.6) -180 (-175) -7.2 (-6.3) 40.0 (35.9) GCCGUGAG - 71.4 (-64.5) -199 (-178) -9.7 (-9.2) 51.4 (50.4) GCGACAUU - 60.6 (-62.6) -170 (-180) -7.9 (-6.7) 43.9 (37.7) UAACUGGC - 62.6 (-57.8) -174 (-158) -8.6 (-8.7) 47.7 (48.9) UCCACUAG - 64.1 (-54.8) -184 (-155) -6.9 (-6.8) 38.6 (38.5) UGCUGUUG - 52.0 (-63.6) -146 (-184) -6.7 (-6.6) 38.3 (37.2) UGUUCGAC - 66.2 (-64.1) -190 (-186) -7.4 (-6.4) 41.3 (36.3) UUACAGCG - 58.4 (-65.7) -166 (-190) -7.0 (-6.9) 39.5 (38.7) UUGGCACC - 56.2 (-65.0) -153 (-179) -8.7 (-9.4) 49.8 (51.3) AUAACUGGC - 60.7 (-66.1) -168 (-182) -8.7 (-9.6) 48.7 (51.9) AUCUAUCCG - 59.9 (-72.3) -171 (-210) -6.8 (-7.0) 38.4 (38.8) CAACAGCAA - 63.3 (-63.7) -177 (-179) -8.7 (-8.2) 47.8 (45.4) CAACAGCAU - 71.0 (-64.2) -200 (-181) -9.0 (-8.1) 48.1 (44.7) CGCUGUUAC - 71.5 (-72.8) -205 (-210) -8.2 (-7.8) 44.2 (42.3) CGCUGUUAG - 67.7 (-76.0) -193 (-221) -8.0 (-7.5) 44.0 (41.0) CUAACAGCG - 70.8 (-69.0) -199 (-195) -9.0 (-8.6) 48.1 (46.5) GCCAGUUAA - 63.1 (-68.4) -179 (-197) -7.7 (-7.3) 42.7 (40.7)

GUAACAGCG - 77.5 (-69.8) -221 (-197) -9.1 (-8.8) 47.8 (47.4) UUAACUGGC - 67.3 (-69.3) -189 (-195) -8.9 (-8.9) 48.3 (47.9) ACGUAUUAUGC - 95.4 (-90.2) -279 (-261) -9.1 (-9.3) 45.6 (46.6) GCAUAAUACGU - 97.3 (-85.1) -282 (-244) -10.0 (-9.4) 48.4 (47.7) AAUGGAUUACAA -90.7 (-93.2) -260 (-267) -10.2 (-10.3) 50.2 (50.1) AUUGGAUACAAA -93.6 (-93.2) -267 (-267) -10.8 (-10.3) 51.9 (50.1) GUCAGGAAUCUG -79.8 (-93.0) -220 (-260) -11.4 (-12.3) 57.4 (57.5) UUGUAAUCCAUU -76.9 (-98.4) -221 (-292) -8.5 (-7.9) 45.4 (41.4) CACAG - 31.3 (-31.1) -90.6 (-91.9) -3.2 (-2.6) 7.2 (2.2) CUGUG - 31.0 (-33.7) -90.6 (-102) -2.9 (-2.1) 4.5 (0.7) CAACCAACCAAC -117 (-84.8) -326 (-231)-15.9 (-13.1) 64.0 (63.0) CUUCCUUCCUUC -73.8 (-98.0) -2 06 (-287) -9.9 (-8.9) 51.9 (44.7) GAAGGAAGGAAG -108 (-90.9) -292 (-244)-17.4 (-15.0) 71.8 (69.3) GUUGGUUGGUUG -112 (-112.8) -311 (-327)-15.5 (-11.4) 64.1 (51.0) [0025] For **4% delta S degrees, **4% delta G degree of a measurement error of Tm(s) is [deltaH degree / 37] **2% **8%. The melting out temperature Tm calculated the total concentration of oligo ribonucleotide and oligodeoxyribonucleotide as 100microM. Based on such measured value, the value of deltaH degree, deltaS degree, and deltaG degree37 over 16 kinds of a JIRIBO nucleotide / dideoxy ribonucleotide base pairs, and an initiation factor was calculated with the nonlinear least square method. The result is the above-mentioned table 1.

[0026] Next, the value of deltaH degree, deltaS degree, deltaG degree37, and Tm over each base pair formation was calculated based on the aforementioned (1) formula using the maximum contiguity parameter by this invention shown in a table 1, and the value of initiation. For example, "AGCCG r (AGCCG)/d (CGGCT) in a table", i.e., complementary base pairs, is expressed by the sum of the following maximum contiguity base pairs.

r (AGCCG)/d(CGGCT) =r(AG)/d(CT)+r(GC) / d -- (GC)+r(CC) / d(GG)+r(CG) / d (CG) -- it is calculated as follows from this using the parameter value of a table 1 enthalpy change deltaH degree accompanying this complementary base pair formation.

deltaH degree=deltaH degree(rAG/dCT)+deltaH degree(rGC/dGC)+deltaH degree (rCC/dGG) +deltaH degree(rCG/dCG)+deltaH degree (initiation) = -9.1-8.0-9.3-16.3+1.9 = -40.8 [0027] Similarly, entropy change deltaS degree, free energy change deltaG degree37, and Tm are calculated as follows using the parameter value of a table 1.

deltaS degree=deltaS degree(rAG/dCT)+deltaS degree(rGC/dGC)+deltaS degree (rCC/dGG) + deltaS degree(rCG/dCG)+deltaS degree (initiation) = -23.5-17.1-23.2-47.1-3.9 = -114.8 **-115 deltaG degree37=deltaG degree(rAG/dCT)+deltaG degree(rGC/dGC) +deltaG degree (rCC/dGG) + deltaG degree(rCG/dCG)+deltaG degree (initiation) = -1.8-2.7-2.1-1.7+3.1 = -5.2 Tm= [{Rln(Ct/4) +deltaS degree} / deltaH degree] -1-273.15 =[{1.9872xln(100x10-6/4)-114.8} / (-40.8x103)] -1-273.15 = 300.3-273.15 = 27.17 **27.2[0028] Thus, the result calculated using the parameter of the table 1 by this invention was shown in the above-mentioned table 4 in a parenthesis. The average error of calculated value and measured value is understood that the maximum contiguity base pair parameter value which is **2.1 degrees C to **7.0% and Tm to 37, and was shown in a table 1 delta G degrees **8.5% to delta S degrees **7.5% to deltaH degree is very effective.

[0029] For the comparison of the parameter value by this invention, and the conventional parameter value, the actual measurement of deltaG degree37, The calculated value using the maximum contiguity base pair parameter shown in a table 1, the calculated value using the parameter [Proc.Natl.Acad.Sci.USA 83 and 9373-9377 (1986)] of Freier, The calculated value using the parameter [30 Biochemistr(ies) and 1097 - 1118 pages (1991)] by Von Hippel is put in order, and it is shown in a table 5.

[0030]

[A table 5]

Oligo ribonucleotide array Measured value This example Freier's Von Hippel's ---------- AGCCG - 5.7 - 5.2 - 6.6 - 7.4 CGGCU - 4.9 - 5.1 - 6.6 - 7.4 GGUGG - 5.2 - 5.4 - 6.3 - 6.2 ACCGCA - 6.4 - 6.4 -8.8 - 9.1 CAAUCG - 3.3 - 2.9 -4.5-5.9 CACGGC - 6.9-7.2 -8.8 - 9.1 CGAUUG -2.6 -2.6-4.5 -5.0CGGUGC -7.0 - 6.9 - 8.8-9.1 CGUGCC - 6.3-6.1 -8.8 - 9.1 GCACCG-6.2 - 6.4 - 8.8 -9.1 GGCACG-7.3 -7.2-8.8-9.1 AAUACCG - 4.7 -5.3 - 6.5-8.4 ACGUAUG - 5.0 - 4.9 - 6.6 - 7.6 AGCUUCA - 4.7 - 4.9 -8.4 -7.1 CACGGCU-8.5 -8.1 - 10.5 - 10.8 CAUACGU - 4.3 - 4.2 - 6.6 - 7.6 GGACUUA - 4.9 -4.9 - 7.6 -6.7 UAAGUCC - 5.4-5.0 -7.6-7.6 UGAAGCU - 6.4-6.2-8.4 -8.0 AAAAAAAA - 3.8 - 3.9-2.9 -6.1 AAGCGUAG - 7.8 -7.6 - 9.5-10.5 AAUCCAGU -6.1-6.2 -9.2 - 9.3 AAUGUCGC - 7.2 -7.4 - 10.0 - 10.5 ACCUAGUC - 6.9 - 7.0 - 10.5 - 9.7 ACGACCUC -8.6 - 8.6-12.0 -11.3 ACUGGAUU - 7.2 - 6.8 - 9.2 -8.4 AGCGUAAG - 7.3 - 7.6 - 9.5 - 10.5AGUCCUGA - 6.7 -7.2-11.4-10.1 CAACAGCA - 7.8-7.2 -10.1 - 9.7 CACGGCUC-9.6 - 9.6 - 12.8-12.5 CGCUGUAA - 7.2 -6.5-9.6-10.5 CUACGCUU -6.8 -6.0 -9.5-9.6 CUAGUGGA -8.3-7.1 -10.2 -9.7 CUCA CGGC -9.6 -9.6 -12.8 -12.5 CUGAGUCC -7.8 -7.2 -11.4 -10.1 CUUACGCU -6.4 -6.0 -9.5 -9.6 GACUAGGU -8.1 -7.6 -10.5 -9.7 GAGCCGUG -9.5-9.2 - 12.8 - 12.5 GAGGUCGU - 9.2 - 8.3-12.0 -11.3 GCCAGUUA - 7.2-6.3 -10.5 - 9.6GCCGUGAG-9.7-9.2 -12.8 - 12.5 GCGACAUU - 7.9 - 6.7-10.0 -9.6UAACUGGC -8.6 -8.7 - 10.5 - 10.5 UCCACUAG -6.9 - 6.8-10.2-9.7UGCUGUUG-6.7 - 6.6 - 10.1 -8.8 UGUUCGAC - 7.4 -6.4 - 10.1 - 8.8 UUACAGCG - 7.0 -6.9 - 9.6-9.6 UUGGCACC - 8.7 - 9.4 - 12.4 -11.2AUAACUGGC - 8.7 - 9.6 - 11.4 - 11.8 AUCUAUCCG - 6.8-7.0 -10.7 - 11.8 CAACAGCAA-8.7 - 8.2 -11.0-11.0 CAACAGCAU - 9.0 - 8.1 - 11.0-11.0 CGCUGUUAC-8.2 - 7.8-11.7 -11.3 CGCUGUUAG - 8.0 -7.5 -11.3 - 11.3 CUAACAGCG -9.0 - 8.6 -11.3-12.2 GCCAGUUAA - 7.7 - 7.3 - 11.4 -10.9 GUAACAGCG - 9.1 -8.8-11.7-12.2 UUAACUGGC - 8.9 - 8.9 -11.4 - 10.9 ACGUAUUAUGC - 9.1 - 9.3 -12.9 -13.5 GCAUAAUACGU - 10.0 -9.4 - 12.9 - 14.4 AAUGGAUUACAA -10.2-10.3 -13.1 - 13.6 AUUGGAUACAAA-10.8 - 10.3 - 13.1 - 13.6 GUCAGGAAUCUG-11.4 - 12.3 -17.3 - 16.1 UUGUAAUCCAUU - 8.5 - 7.9 - 13.1 - 12.7CACAG - 3.2 -2.6-4.0 -3.8 CUGUG - 2.9 - 2.1 - 4.0 - 3.8 CAACCAACCAAC -15.9-13.1 -16.8-16.9 CUUCCUUCCUUC - 9.9 -8.9 -17.1 - 14.2 GAAGGAAGGAAG -17.4 -15.0 -17.1 -16.9GUUGGUUGGUUG -15.5 -11.4 -16.8 -14.2 Average error (%) 7.0 24.5 24.6 [0031] clear from a table 5 -- as -- the former Freier the forecast using a parameter -- and -- Von Hippel The average errors of the forecast and actual measurement using a parameter are 24.5% of each, and 24.6%. On the other hand, it turns out that the value predicted by the method of this invention using the parameter of a table 1 shows coincidence with very good both if an average error with an actual measurement is 7.0% and takes into consideration an experimental error (**8 - 10%).

[0032] Moreover, even if it uses the value within the limits shown in said table 2 instead of the value shown in a table 1, although precision falls a little, it can predict the stability of the complementary base pair structure of oligo ribonucleotide / oligodeoxyribonucleotide in the precision which should be satisfied enough. Or even if it uses the value of the **10% of within the limits focusing on the value shown in a table 1, the stability of the complementary base pair structure of oligo ribonucleotide / oligodeoxyribonucleotide can be predicted in the precision which should be satisfied enough.

[0033]
[Effect of the Invention] According to this invention, stability in case oligo ribonucleotide and oligodeoxyribonucleotide [complementation / it] form complementary base pair structure can be predicted, and this is useful to development of the physic based on a nucleic acid, a diagnostic drug and detection medicine, or those methods.

[Translation done.]

* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] Drawing which plotted the experimental result about the duplex chain of an octamer which consists of r (CUUACGCU)/d (AGCGTAAG), and the duplex chain of an octamer which consists of r (CUACGCUU)/d (AAGCGTAG).

[Translation done.]

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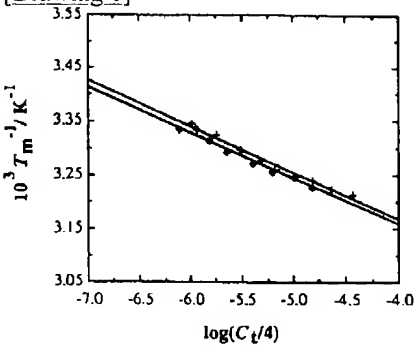
DRAWINGS

[Drawing 1]



[Translation done.]